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APPLICATION NUMBER: *60/425,661*
FILING DATE: *November 13, 2002*
RELATED PCT APPLICATION NUMBER: *PCT/US03/36292*

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60425661 .111302 PROV

jc923 U.S. PTO 11/13/02

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jc923 U.S. PTO 11/13/02 60/425661

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Gaetano	Montelione	Piscataway, NJ

☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

A Process for Designing Inhibitors of the Life Cycle of Influenza A Virus Targeted to the Viral Non-Structural Protein 1

Direct all correspondence to: CORRESPONDENCE ADDRESS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 16 ☐ CD(s), Number

☐ Drawing(s) *Number of Sheets* ☐ Other (specify)

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

		FILING FEE AMOUNT ($)
☒	Applicant claims small entity status. See 37 CFR 1.27.	$80.00
☐	A check or money order is enclosed to cover the filing fees	
☒	The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 182362	
☐	Payment by credit card. Form PTO-2038 is attached.	

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☒ Yes, the name of the U.S. Government agency and the Government contract number are: NIH R01 GM47014

Respectfully submitted,

SIGNATURE _____ Date 11/12/02

TYPED or PRINTED NAME Vincent Smeraglia REGISTRATION NO. *(if appropriate)* 44,340

TELEPHONE 732 932-0115 ext. 3021 Docket Number: 03-053

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

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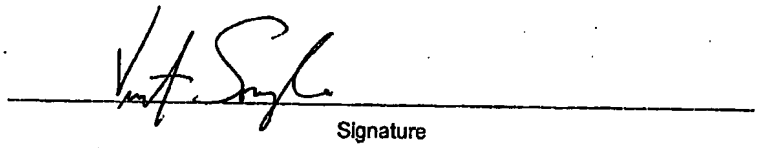

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Vincent Smeraglia
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Provisional Patent Application

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G. T. Montelione
October 5, 2002

!!!!!Imminent Publication!!!!!

RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY
Disclosure Form Summary

For office use only

Docket Number _____ **Date Disclosed**_____

G. T. Montelione October 5, 2002

1. Disclosure Title:

A Process for Designing Inhibitors of the Life Cycle Of Influenza (Flu) Virus Targeted to the Viral Non-Structural Protein 1

2. Relation to Previous Disclosure: Yes ____ No *X*
If Yes, provide disclosure number and title: _____

3. Federal Funding and Other Agreements:

Funding:
NIH/Government Yes_X_ No_____ Grant No. NIH RO1 GM47014

Corporate Sponsor Yes____ No _X Sponsor Name(s) _____

Center Funds Yes ____ No _X_ Name of Center _____

Other Yes X No ___ Source NIH Grant AI11772 to Dr. R. Krug, University of Texas

Related Agreements:
Was the research that produced this invention performed in whole or in part under any of the following agreements? Please check all that apply. Please provide copies of all agreements when possible.
None apply.

Sponsored Research Agreement ___	Material Transfer Agreement ___	Consulting Agreement___
Collaborative Research Agreement ___	Inter-Institutional Agreement ___	
Grant-in-aid ___	Service Agreement ____	

Materials:
Did you use any material obtained from another party in developing this technology (e.g. plasmids, antibodies, computer software)?

G. T. Montelione
October 5, 2002

Yes ____ No X Source: All reagents were generated at Rutgers Univ or Univ of Texas, Austin

4. Critical Dates:

Disclosure or presentation to others? Please include impending disclosures or presentations.
Yes X No ___

To Whom/Affiliation: The invention has been described verbally on Sept. 17, 2002 to PTC Therapeutics. Inc. (http://www.ptcbio.com/big/indexhome.html), a biotechnology company in the Piscataway area, who have expressed strong interest in commercializing the information, concepts, and experimental design outlined in this disclosure. In the next few days, Dr. Krug plans to provide to PTC Therapeutics, Inc a written version of the disclosure in order to develop a joint research proposal to carry out the plan outlined in the attached Intellectual Property Disclosure.

Submitted abstract or manuscript? Yes _X_ No ___

Expected publication journal and date: Some background work related to this disclosure has already been published. Part of the results outlined in this disclosure will be submitted in the next few days to the journal Biochemistry.

Submitted in grant application form? Yes _X_ No ___
Agency and expected funding date: We plan to submit a Letter of Intent for Grant Proposal outlining this concept to a NIH RFA in Bioterrorisim on October 25, 2002. This letter of intent need not provide an enabling disclosure. We will then submit a full grant proposal in mid November, which will be an enabling disclosure.

Published in any form – including internet? Yes___ No _X__
Where and when_____

5. Contributors

Name(print)	School & Dept (or Institution, if other than Rutgers)	Phone No.	Email
1. Gaetano T. Montelione [Primary Rutgers Contact]	CABM, Rutgers University	732-235-5321	guy@cabm.rutgers.edu
2. Robert Krug	University of Texas, Austin, TX	512-232-5563	rkrug@intron.icmb.utexas.edu

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03-053

G. T. Montelione
October 5, 2002

CONFIDENTIAL

OFFICE OF CORPORATE LIAISON AND TECHNOLOGY TRANSFER

TECHNOLOGY DISCLOSURE FORM

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INSTRUCTIONS. This form will be used by the Office of Corporate Liaison and Technology Transfer to establish a record of your disclosed technology and to begin the processes of 1) determining whether to patent or otherwise protect the technology, and 2) attempting to license it to industry. We consider this a joint venture between you and OCLTT, which will succeed best if we work closely together. Please complete and return it to the Office of Corporate Liaison and Technology Transfer. It will promptly be assigned a case number and assigned to a Licensing Manager.

A. GENERAL QUESTIONS.

1. Please indicate the type(s) of technology associated with this disclosure (check all that apply):
   - X possibly patentable invention (utility or design)
   - _____ software
   - _____ copyrighted work (non-software)
   - X proprietary material or information
   - _____ trademark associated with the above

2. Title of that technology. Please provide a brief descriptive title for the invention or technology.

A Process for Designing Inhibitors of the Life Cycle Of Influenza A Virus

Targeted to the Viral Non-Structural Protein 1

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3. Description. Please provide a brief description of the technology.

Based on a combination of published and unpublished information from the laboratories of Prof Gaetano Montelione (Rutgers University, Piscataway) and Robert Krug (Univ. of Texas, Austin, Texas), we describe an approach for developing assays for the interactions of the "Non Structural Protein 1" (NS1) of influenza virus (flu virus) and its RNA targets. Data from our laboratories demonstrate specific sites in the 3D structure of NS1 which if mutated or blocked by small molecules will suppress the viability of the virus. These assays would form the basis for high throughput screening to identify small molecule inhibitors of the interactions between NS1 and RNA, which would be lead compounds for antiviral drug development.

4. Sponsorship. Was the work that led to the technology sponsored? If so, please describe by whom, and attach a copy of the sponsorship agreement or the ORSP/OCLTT reference number.

Sponsor: **The National Institutes of Health**

Contract or Grant Number: **GM47014 (to GTM at Rutgers) and AI11772 (to RK at Univ of Texas)**

B. POSSIBLY PATENTABLE INVENTIONS Publication, or public presentation of an invention before filing a patent application will permanently bar you and Rutgers from obtaining certain patent rights. Therefore, it is imperative that you work closely with OCLTT before publishing enabling information about your invention.

1. Basic Invention Information. Please answer the following basic questions.

a. What was the earliest date, time, and place the invention was conceived? By whom was it conceived?

The proposal outlined in the attached Intellectual Property Disclosure for developing an assay suitable for high throughput screening was made in a discussion between Drs Montelione and Krug in Dr Montelione's office on August 5, 2002. The concept was conceived jointly. The plan was extended in phone discussions on August 28, 002, and in further phone discussions during the first two weeks (Sept 1 – 14, 2002) of September 2002.

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b. What was the date and location of the first sketch, drawing, or photo? (Attach a copy).

A sketch was made on Dr. Montelione's blackboard and discussed at the meeting on August 5, 2002. No hard copy of this sketch is available.

c. What was the date, place, and identification of its first written description? (Attach a copy).

Many of the underlying concepts of this proposal are outlined in a manuscript that was prepared over a two-and-one half year period beginning in the spring of 1999 and ending in October 2002. The first written description of the proposed process for high throughput screening was made by Dr. Montelione on Sept. 26, 2002. This description was refined by Drs. Krug and Montelione over the next few days. This Intellectual Property Disclosure is attached.

2. Commercial Utility, Novelty, and Interest.

a. How important do you think this technology might be from a commercial perspective and why?

It forms the basis for the identification and optimization of lead compounds for drug development that would be useful in the treatment of flu infection. This is an important market for pharmaceutical drug development.

b. What are its principal novel and unusual features?

We have (i) identified for the first time the atoms in the 3D structure of a flu protein, NonStructural Protein 1, which are essential for its ability to bind target RNA molecules as part of the flu life cycle, (ii) identified for the first time the atoms in the structure of a flu protein, NonStructural Protein 1, which are essential for its viability in infected cells, (iii) we have used this information to design an assay for NS1-RNA interactions suitable for high throughput screening, and (iv) we have designed an approach for high throughput screening that will allow discovery and optimization of small molecule lead compounds suitable for further drug development

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c. What commercial advantages does it offer over existing technologies? What problems does it solve which would make people want to buy it?

The full set of information needed to demonstrate the critical role of these atoms in the molecular recognition process and in the robustness of flu infection are not yet known in the public domain. Thus, the combination of published information together with this proprietary information described in the attached Intellectual Property Disclosure provides both the motivation and path to a successful assay and high throughput screening approach for identifying lead compounds and eventually antiviral drugs.

d. What companies have expressed an interest in this technology? What other companies might be interested in it?

The proprietary information outlined in this Intellectual Property Disclosure has been discussed with PTC Therapeutics, Inc (http://www.ptcbio.com/big/indexhome.html), a biotechnology company in the Piscataway area, who have expressed strong interest in commercializing the concepts outlined in this disclosure. Dr. Krug plans to provide PTC Therapeutics a copy of the attached Intellectual Property Disclosure imminently in order to develop a plan for a research grant proposal. We request that Rutgers establish a Non Disclosure Agreement with PTC as soon as possible in order to allow the discussions to continue.

e. Do you have an interest in forming or joining a startup company based on this technology?

Not at this time.

3. Experimental Verification.

a. Have you tested the invention experimentally? If so, describe how below.

Yes. See attached Intellectual Property Disclosure.

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b. Have you constructed a prototype, model, or test samples which are available for examination? If so, when and where? (Provide a copy or photo)

Yes. Will provide this information at a later date.

4. Descriptions and Publications.

a. Please attach or send to OCLTT a copy of all relevant drawings, descriptions, publications, abstracts, overheads, slides, experimental records and notes pertaining to your technology or invention and your presentation(s) and publication(s) relating to it.

Will provide this information at a later date.

b. Have you described the invention in any abstracts, theses, publications or presentations or do you plan to do so? What was/are the date(s) and nature of the actual and/or planned publication(s) or presentation(s)?

Though some aspects of our understanding of the NS1A-RNA interaction and its importance to the viability of the virus have been published, the process and reagents for drug design outlined here have not been published or disclosed except in our discussion with PTC Therapeutics on Sept. 17, 2002.

5. References. Are there publications or presentations by others that are related to this technology which might be helpful to our evaluation? If so, please provide a copy or a reference to us.

Will provide this information at a later date.

6. Notebooks. It is important for the legal process of obtaining a patent or in otherwise protecting your invention to keep up-to-date and accurate records which are maintained and witnessed in a bound, sequentially page numbered, research notebook on a daily basis. The notebook should demonstrate when you conceived of the invention, and that thereafter, you were persistent in your efforts to reduce your invention to practice.

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a. Have you kept an accurate, daily notebook of your progress and work on this invention? (Provide a copy of relevant pages).

Yes

b. Have you signed each day's entry in the notebook?

No. But much of the information and intermediate versions of manuscripts are date stamped by our computer systems.

c. Has a witness not involved with the invention but able to understand it also signed off on each day's entry into the notebook?

No

7. Inventor(s). Inventorship is a matter of patent law and will be determined by a patent attorney. Under the US patent law, inventors must make original contributions to the concept or reduction to practice of the invention. Merely carrying out experiments at the direction of another, or supplying a research reagent, or reviewing data does not amount to inventive contribution. Applying these criteria, please list all of the co-inventors of this invention and have them thereon sign and complete this form at the end. NOTE that the authors of publications may be different from inventors of patentable inventions.

1. Gaetano T. Montelione CABM, Rutgers University, Piscataway, NJ
2. Robert M. Krug University of Texas, Austin, Texas

8. Rutgers Patent Policy Distributions of Royalty Income. Under Rutgers' Patent Policy, if a patent application (or resulting issued patent) results from the technology described in this disclosure, the inventors listed on such patent application or patent will receive equal shares of any royalty income. However, it is possible for the inventors to agree among themselves that the inventors' share of royalty income is to be divided differently. The inventors may also agree that royalty income should be shared with additional persons who may have been involved in development of the invention but who do not satisfy the legal requirements for inventorship. If you do not wish the normal equal distribution among inventors under the patent policy, please provide OCLTT an agreement signed by all inventors, which indicates the mutually agreed alternative distribution.

C. SOFTWARE. Software may be protected by several types of intellectual property, including patents, copyrights, trade secrets, and related trademarks. OCLTT will work with you to determine which one(s) are most appropriate.

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not applicable

D. COPYRIGHTED WORKS. Copyright protection automatically extends to the actual expression of the work into a tangible medium (e.g., a writing, a program, a picture) and not the concept of the technology. This protection can be enhanced by registration with the Federal government.

not applicable.

E. PROPRIETARY MATERIALS, TECHNICAL INFORMATION, OR TRADE SECRETS. In order to maintain the value of this information or material, they may not be made available to anyone unless they sign an appropriate Confidentiality Agreement with OCLTT.

1. Please describe any proprietary materials or information which you believe may be protectable and of commercial value.

See attached Intellectual Property Disclosure

F. TRADEMARKS, TRADENAMES, DOMAIN NAMES. Trademarks are associated with classes of goods or services and tradenames can identify the source of goods and services. Domain names reserve addresses on the World Wide Web. These marks and names can be protected and licensed to companies interested commercializing products and services based on Rutgers invented technology. Please identify any trademarks you would like to be used in connection with any of the above mentioned technologies, and any steps you have taken to register them.

Not applicable

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G. INVENTOR/AUTHOR/CREATOR INFORMATION AND SIGNATORIES

G. T. Montelione
October 5, 2002

PRIMARY INVENTOR AUTHOR, OR CREATOR OF THE TECHNOLOGY DESCRIBED IN THIS FORM (PI)

(This person will interact with this office, will keep the others listed on this form informed of all such interactions, and is usually a University Professor).

Name of PI: Gaetano Montelione

Social Security Number of PI: 124-52-4880

PI's Department: Molecular Biology and Biochemistry, FAS

PI's Budget Unit: Center for Advanced Biotechnology and Medicine, Rutgers University

PI's Research Unit: Center for Advanced Biotechnology and Medicine, Rutgers University

PI's Research Unit Director/Chairperson: Prof. Aaron Shatkin

PI's Title: Prof. of Molecular Biology and Biochemistry,
Rutgers University

PIs Work Address: CABM-Rutgers University, 679 Hoes Lane, Piscataway, NJ 08854

PI's Work Phone: 732-235-5321 Home: unlisted

PI's Home address: 127 N. Fifth Ave, Highland Park, NJ 08904

Signature:_____ Date:_____

OTHER POSSIBLE INVENTORS, AUTHORS OR OWNERS OF THE TECHNOLOGY

Name: Robert M. Krug

Social Security Number:_____

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G. T. Montelione
October 5, 2002

Department: Institute for Cellular and Molecular Biology, Section of Microbiology and Molecular Genetics, University of Texas at Austin, Austin, TX 78746

Budget Unit: Not applicable
Research Unit: Not applicable
Research Unit Director/Chairperson: Not applicable

Title: Professor of Microbiology and Molecular Genetics

Work Address:_____

Work phone: 512-232-5563 Home_____

Home Address:_____

Signature:_____Date:_____

H. PLEASE HAVE THIS FORM WITNESSED BY SOMEONE WHO KNOWS AND UNDERSTANDS THE TECHNOLOGY.

WITNESS:_____

PRINTED NAME:_____

I. PLEASE HAVE THE DEPARTMENTAL CHAIR AND THE DIRECTOR OF THE PI's RESEARCH UNIT SIGN THIS FORM AND PROVIDE A COPY TO HIM.

Department Chair:_____

Date:_____

Director of Research Unit:_____

Date:_____

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CONFIDENTIAL

Intellectual Property Disclosure

September 26, 2002

A Process for Designing Small Molecule Inhibitors of the Life Cycle of Influenza (Flu) Virus Targeted to the Viral Non-Structural Protein 1

Gaetano Montelione[1] and Robert Krug[2]

[1]Center for Advanced Biotechnology and Medicine, and Dept. of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NY

[2]Institute for Cellular and Molecular Biology, Section of Microbiology and Molecular Genetics, University of Texas at Austin, Austin, TX 78746

The influenza virus non-structural protein 1 encoded by influenza A virus (NS1A protein) is a multifunctional protein involved in both protein-protein and protein-RNA interactions. NS1A binds non-specifically to double-stranded RNA (dsRNA) and to specific protein targets, and regulates several post-transcriptional processes. The N-terminal structural domain corresponding to the first 73 amino acids of the NS1 protein from influenza A/Udorn/72 virus [NS1A(1-73)] possesses all of the dsRNA-binding activities of the full-length protein (Qian, et al. 1995). Both NMR and X-ray crystallography of this domain have demonstrated that it is a head-to-tail symmetric homodimer which forms a six-helical chain fold (Chien et al., 1997; Liu et al., 1997), a unique structure that differs from that of the predominant class of dsRNA-binding domains, referred to as dsRBMs, that are found in a large number of eukaryotic and prokaryotic proteins. Each polypeptide chain of the NS1A(1-73) domain dimer consists

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of three α-helices, corresponding to the segments Asn^4-Asp^{24} (helix 1), Pro^{31}-Leu^{50} (helix 2), and Ile^{54}-Lys^{70} (helix 3).

Biophysical experiments on complexes containing NS1A(1-73) and a short 16-base-pair synthetic dsRNA duplex have been carried out at Rutgers University and the University of Texas at Austin. From sedimentation equilibrium measurements we determined that the dimeric NS1A(1-73) binds to the dsRNA duplex with a 1:1 stoichiometry, yielding a complex with an apparent dissociation constant (K_d) of ≈1 μM. Circular dichroism and nuclear magnetic resonance (NMR) data demonstrate that the conformations of both NS1A(1-73) and dsRNA in the complex are similar to their free forms, indicating little or no structural change of the protein or RNA upon complex formation. NMR chemical shift perturbation experiments show that the dsRNA-binding epitope of NS1A(1-73) is associated with helices 2 and 2′. Analytical gel filtration and gel shift studies of the interaction between NS1A(1-73) and different double-stranded nucleic acids indicate that NS1A(1-73) recognizes canonical A-form dsRNA, but does not bind to dsDNA or dsRNA-DNA hybrids, which feature B-type and A/B-type intermediate conformations, respectively. On the basis of these results, we propose a three-dimensional model of the complex in which NS1A(1-73) sits astride the minor groove of A-form RNA with a few amino acids in the helix 2/helix 2′ face forming an electrostatically stabilized interaction with the phosphodiester backbone. This mode of dsRNA binding differs from that observed for any other dsRNA-binding protein.

The proposed structure of the complex confirms site-directed mutagenesis studies (Wang et al., 1999) at Rutgers University and Univ of Texas at Austin, which were guided by the NMR and X-ray crystal structures (Chien et al., 1997; Liu et al., 1997), demonstrating that the basic side chains of four residues on the surface of NS1A(1-73) are required for dsRNA binding, residues Arg 38, Arg 38', Lys 41, and Lys 41' (Wang et al., 1999). Mutation of these residues to alanine results in a dimeric NS1(1-73) molecule which has no detectable dsRNA-binding activity, based on gel shift assays. Utilizing the recently developed reverse genetic system, whereby influenza viruses can be generated

by transfection of multiple DNAs without a helper virus (Fodor et al, 1999; Neumann et al,1999), we generated at Univ. of Texas a recombinant influenza A virus encoding a NS1A protein containing these mutations in its RNA-binding domain. This virus is highly attenuated, and in fact does not form visible plaques. These data demonstrate that the interaction between NS1A and its RNA targets involving the specific surface binding epitope including residues Arg 38, Arg 38', Lys 41, and Lys 41' are ***essential for the viability of the virus.***

From these studies we propose the following process for discovering, designing, and optimizing inhibitors of influenza virus:

1. Molecules discovered or designed to interfere with the interactions between the NS1A protein and its RNA substrates would be important lead compounds for the design of influenza antiviral drugs. In particular, compounds directed to the characterized specific surface binding epitope including residues Arg 38, Arg 38', Lys 41, and Lys 41' of the NS1A protein would be good candidates as lead compounds for antiviral drug design. Such compounds should also be useful lead compounds for the development of antiviral drugs directed against all strains of influenza A virus, as well as strains of the influenza B virus which have a similar dsRNA-binding epitope.

2. These compounds would be particularly valuable for the development of protective agents to defend against bioterrorism using influenza A (or B) virus as the biological weapon.

3. A high throughput *in vitro* assay (HTP-Assay) can be developed to measure the affinity of binding various synthetic dsRNA substrates and full length NS1A and/or the NS1A(1-73) RNA binding domain (the NS1 Targets). This assay would use either or both of the standard methods of fluorescence resonance energy transfer (FRET) or fluorescence polarization with tagged dsRNA molecules, NS1A, or S1A(1-73) molecules

to monitor interactions between these protein targets and various dsRNA duplexes (RNA Substrates), and to measure binding affinities.

4. The one or more HTP-Assays will be used to screen compound libraries using conventional high-throughput screening technologies to identify molecules which inhibit the interactions between the NS1 Targets and the RNA Substrates. These compound libraries will be obtained through a collaboration with one or more biotechnology companies with expertise in this area, or purchased from commercial sources.

5. This process would use both random compound libraries and biased compound libraries designed using the particular structural features of the known NS1 Target - RNA Substrate interaction sites that have been deduced from our published (Chien et al., 1997; Liu et al., 1997; Wang et al, 1999) and proprietary structural data.

6. Binding sites on the surface of NS1A(1-73) of small molecule inhibitors identified by HPT screening can be characterized using chemical shift perturbation NMR experiments, together with the complete set of NMR resonance assignments that we have determined for this protein domain. Resonance assignments can also be obtained for RNA Substrates, and the binding sites of small molecule inhibitors on the surface of RNA Substrates caln also be characterized by NMR. The locations of binding sites will provide data for linking together multiple initial inhibitor leads and for optimizing lead design.

7. Having identified inhibitors of the interaction between NS1Targets and RNA Substrates, the inhibitors will be tested for their ability to inhibit influenza A virus replication in tissue culture experiments. We will determine the lowest concentration of each inhibitor that effectively inhibits influenza virus replication, using both high and low multiplicities of infection. We will identify the most effective inhibitors of virus replication for subsequent animal experiments.

References

Chien, C.-Y.; Tejero, R.; Huang, Y.; Zimmerman, D.; Krug, R.M.; Montelione, G.T. **Nature Struct. Biol.** 1997, 4: 891 - 895. A novel RNA-binding motif in influenza A virus non-structural protein 1.

Fodor, E.; Devenish, L.; Engelhardt, O. G.; Palese, P.; Brownlee, G. G.; Garcia-Sastre, A. **J. Virol.** 1999, 73: 9679-9682. Rescue of influenza A virus from recombinant DNA.

Liu, J.; Lynch, P.; Chien, C.-Y.; Montelione, G.T.; Krug, R.M.; Berman, H. **Nature Struct. Biol.** 1997, 4: 896 - 899. Crystal structure of the N-terminal fragment of NS1 protein from influenza A virus: A multifunctional RNA-binding domain.

Neumann, G.; Watanabe, T.; Ito, H.; Watanabe, S.; Goto, H.; Gao, P.; Hughes, M.; Perez, D. R.; Donis, R.; Hoffmann, E.; Hobom, G.; Kawaoka, Y. **Proc. Natl. Acad. Sci. U.S.A.** 1999, 96: 9345 – 9350. Generation of influenza A viruses entirely from cloned cDNAs.

Qian, X.-Y.; Chien, C.-Y.; Lu, Y.; Montelione, G.T.; Krug, R.M. **RNA** 1995, 1: 948-956. An amino-terminal polypeptide fragment of the influenza virus NS1 protein possesses specific RNA-binding activity and largely helical backbone structure

Wang, W.; Riedel, K.; Lynch, P.; Chien, C.-Y.; Montelione, G. T.; Krug, R. **RNA** 1999, 5: 195 - 205. RNA-binding by the novel helical domain of the influenza virus NS1 protein requires its dimeric structure and a small number of specific basic amino acids.

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